Early mechanical alterations in phospholamban mutation carriers: identifying subclinical disease before onset of symptoms

Brief title: Early mechanical alterations in PLN mutation carriers

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ABSTRACT

Objectives: We aimed to explore echocardiographic characteristics of phospholamban (PLN) p.Arg14del mutation carriers to investigate whether structural and/or functional abnormalities can already be identified before onset of symptoms.

Background: Carriers of the genetic PLN p.Arg14del mutation may develop arrhythmogenic and/or dilated cardiomyopathy. Overt disease is preceded by a presymptomatic phase of variable length in which disease expression seems to be absent.

Methods: PLN p.Arg14del mutation carriers with an available echocardiogram were included. Mutation carriers were classified as presymptomatic if they had no history of ventricular arrhythmias (VA), premature ventricular complex count <500/24h and left ventricular (LV) ejection fraction ≥45%. Additionally, we included 70 control subjects with similar age and sex distribution as presymptomatic mutation carriers. Comprehensive echocardiographic analysis (including deformation imaging) was performed.

Results: The final study population comprised 281 PLN p.Arg14del mutation carriers of which 139 were classified as presymptomatic. In comparison to control subjects, presymptomatic mutation carriers had lower global longitudinal strain and higher LV mechanical dispersion (both p<0.001). Additionally, post-systolic shortening (PSS) in the LV apex was observed in 43 presymptomatic mutation carriers (31%) and in none of the control subjects. During a median follow-up duration of 3.2 [IQR 2.1-5.6] years in 104 presymptomatic mutation carriers, nonsustained VA occurred in 13 (13%). Presence of apical PSS was the strongest echocardiographic predictor of VA (multivariable hazards ratio 5.11 [95% confidence interval (CI): 1.37-19.08], p=0.015), resulting in a negative predictive value of 96% [95% CI: 89-98%] and a positive predictive value of 29% [95% CI: 21-40%].

Conclusions: Global and regional LV mechanical alterations in PLN p.Arg14del mutation carriers precede arrhythmic symptoms and overt structural disease. Presymptomatic mutation carriers with normal deformation patterns in the apex are at low risk of developing VA within three years, whereas mutation carriers with apical PSS appear to be at higher risk.

Key words: phospholamban, family screening, early detection, risk stratification, echocardiography, deformation imaging

ABBREVIATIONS:

ARVC	arrhythmogenic right ventricular cardiomyopathy
DCM	dilated cardiomyopathy
GLS	global longitudinal strain
LV	left ventricle/ventricular
LVEF	left ventricular ejection fraction
LVMD	left ventricular mechanical dispersion
PLN	phospholamban
PSS	post-systolic shortening
PVC	premature ventricular complex
RV	right ventricle/ventricular
RVFWS	right ventricular free-wall strain
VA	ventricular arrhythmia

Introduction

Phospholamban (PLN) is a phosphoprotein in the sarcoplasmic reticulum which plays a key role in cardiac contraction and relaxation through regulation of calcium homeostasis in cardiomyocytes (1). Various cardiomyopathy-related mutations have been described in the gene that encodes PLN (2). One mutation of particular interest is p.Arg14del, which was identified in a large subset of arrhythmogenic right ventricular cardiomyopathy (ARVC) and dilated cardiomyopathy (DCM) patients in the Netherlands (3). To date, this founder mutation is the most prevalent single cardiomyopathy-related mutation in the Netherlands (4). Besides the Netherlands, this mutation has also been identified in several other European countries and North America (5–9).

Family members of index patients with PLN p.Arg14del routinely undergo genetic cascade screening. This strategy has led to the identification of a large number of presymptomatic individuals (i.e. without phenotypic expression) carrying this mutation (10). Repeated cardiac screening is necessary in this group because of their risk of developing malignant ventricular arrhythmias (VA) and/or heart failure (11,12). Disease penetrance seems to be age-dependent with symptoms often beginning around the fifth decade (3,13,14). However, optimal follow-up frequency and timing of therapeutic intervention is still a major challenge in presymptomatic individuals because of incomplete disease penetrance and variable disease expressivity. Early identification of high-risk mutation carriers, even before the onset of symptoms, would enable close monitoring and early therapeutic intervention to prevent life-threatening VA and/or slow down progression of heart failure.

A promising technique in this regard is echocardiographic deformation imaging. This non-invasive technique allows quantification of global and regional myocardial function and has previously been shown to reveal early signs of disease in several cardiomyopathies (15–20). Accordingly, we hypothesize that dysregulation of intracellular calcium handling in PLN

p.Arg14del mutation carriers leads to a change in myocardial mechanical behavior in early stages of disease, which may be detected by echocardiographic deformation imaging.

In the present study we aimed to explore echocardiographic characteristics in PLN p.Arg14del mutation carriers to investigate whether structural and/or functional abnormalities can already be identified before onset of symptoms. Additionally, we aimed to evaluate the prognostic value of echocardiographic measurements in presymptomatic mutation carriers.

Methods

This observational retrospective longitudinal cohort study was approved by the institutional medical ethics committee of the University Medical Center Utrecht and complied with the Declaration of Helsinki and the European General Data Protection Regulation.

Study population

Study participants were derived from a national registry in which all carriers of mutations associated with arrhythmogenic cardiomyopathy are enrolled, regardless of their phenotype (21). For the current study, we randomly included 350 PLN p.Arg14del mutation carriers (≥18 years) who underwent transthoracic echocardiography between 2006 and 2019 at the one of the three main including centers: University Medical Center Utrecht, University Medical Center Groningen or Amsterdam University Medical Center. This group of mutation carriers consisted of both index patients and relatives. Subjects were excluded in case of an incomplete echocardiogram or an echocardiogram of suboptimal quality (explained in detail below). Subjects were also excluded in case they had other relevant cardiovascular disorders as shown in **Figure 1**. If a subject had more than one available echocardiogram, the first one with appropriate image quality was used for this study.

In addition to mutation carriers, we included 70 control subjects. This control group consisted of (i) healthy non-athlete subjects who were recruited for a previous study and (ii) subjects who were found to have no cardiovascular disease after comprehensive screening at the Cardiology outpatient clinic (22).

Cardiac evaluation

We first evaluated the medical history of PLN p.Arg14del mutation carriers until the echocardiographic examination, with regard to (i) occurrence of (non)sustained VA, (ii) premature ventricular complex (PVC) count on Holter recordings and (iii) left ventricular ejection fraction (LVEF) by cardiac imaging (echocardiography or cardiovascular magnetic resonance imaging (CMR)). Nonsustained VA was defined as ventricular tachycardia of at least three beats with a frequency of at least 100 beats per minute, lasting not more than 30 seconds. Sustained VA was defined as ventricular tachycardia lasting more than 30 seconds, ventricular fibrillation or appropriate implantable cardioverter defibrillator (ICD) intervention.

According to the 2010 Task Force Criteria for ARVC, a cut-off value of 500 PVCs per 24 hours was used (23). According to previous studies and an ongoing interventional trial (clinicaltrials.gov identifier: NCT01857856), a cut-off value of 45% was used for LVEF (13,24). On the basis of these data, mutation carriers were classified into the following three groups:

- Presymptomatic stage: no history of VA, PVC count <500/24h, LVEF ≥45%
- Arrhythmic stage: history of (non)sustained VA and/or PVC count ≥500/24h, LVEF ≥45%
- Structural stage: LVEF <45%

In mutation carriers who had no history of VA and a normal LVEF, but no available Holter recording (until one month after the echocardiogram), we evaluated cardiac symptoms (palpitations or suspected cardiac (pre-)syncope) as reported by the treating physician. The absence or presence of these symptoms was used to classify these mutation carriers into the presymptomatic or into the arrhythmic stage, respectively.

In addition to the aforementioned data, we evaluated the New York Heart Association (NYHA) Functional Classification as per judgement of the treating physician. Standard 12-lead electrocardiograms (ECG) that were recorded within one year before or after the echocardiogram were evaluated for the presence of low QRS voltages and T-wave inversions. Low QRS voltages were considered present in case QRS peak-to-peak amplitudes in leads I, II, and III were <0.5 mV or when the amplitudes in all precordial leads were <1.0 mV. T-wave inversions were assessed in the precordial leads V_2-V_6 and in the inferior leads II, III, aVF, in the absence of a left- or right bundle branch block (LBBB/RBBB). Contrast-enhanced CMR studies that were performed within one year before or after the echocardiogram were categorized according to presence or absence of late gadolinium enhancement (LGE) (24).

Conventional echocardiography

All echocardiograms were performed with Vivid 7, E9 and E95 machines (GE Healthcare, Horten, Norway) as part of routine clinical care. Echocardiograms were retrieved and uploaded to the University Medical Center Utrecht core lab. All measurements were performed by one experienced operator who was blinded for clinical data during the measurements. Conventional echocardiographic measurements were performed in

accordance with current recommendations (25). Details on these measurements are provided in the **supplemental methods**.

Deformation imaging

Longitudinal strain analysis was performed by 2D-speckle tracking with EchoPAC version 203 (GE Healthcare, Horten, Norway). More details on post-processing methods are provided in the **supplemental methods** and in a previous study (26). As for the standard echocardiographic measurements, deformation analyses were performed by one experienced operator who was blinded for clinical data during the measurements.

Global deformation imaging

For LV deformation imaging, apical 4-, 2- and 3- chamber views were used (27). Images with a frame rate <50/s, foreshortened images or images requiring exclusion of more than one segment were not used for analysis. Global longitudinal strain (GLS) reflects global LV myocardial function and was defined as the average global peak strain (%) from the three apical views. GLS values are reported as absolute values. LV mechanical dispersion (LVMD) reflects heterogeneity in LV contraction and was defined as the standard deviation of time to peak longitudinal strain (in ms) from the 18 LV segmental deformation curves.

Right ventricular (RV) deformation imaging was performed when an RV-focused apical 4-chamber view was available (28). Single-wall analysis was performed on the RV free wall. Timing of pulmonic valve closure was derived from spectral Doppler recordings in the parasternal short-axis view. RV free-wall strain (RVFWS) reflects global RV myocardial function and was defined as the average systolic peak strain (%) from the three RV free-wall segments (basal, mid and apical). RVFWS values are reported as absolute values.

Regional deformation imaging

The 18 regional LV deformation curves were evaluated for presence of post-systolic shortening (PSS), which was defined as longitudinal myocardial shortening after aortic valve closure in any of the LV segments (**supplemental figure 1**) (29). Timing of aortic valve closure was derived from the 2D-recording in the apical 3-chamber view. The degree of PSS was expressed using the post-systolic index, which is the amount of additional negative strain after aortic valve closure as a percentage of the peak strain in one particular segment. In this study, PSS was considered present when the post-systolic index was ≥10% in one particular segment (29). For assessment of predilection sites of PSS, we used the LV models that are automatically computed by the software package.

Follow-up data

Follow-up data was derived from an electronic research data platform after performing all echocardiographic measurements (21). To investigate the prognostic value of echocardiographic measurements in presymptomatic mutation carriers, we evaluated whether these individuals developed sustained or nonsustained VA during follow-up (i.e. at least one month after the date of echocardiographic examination). Since arrhythmia surveillance in these mutation carriers is usually performed by Holter recording, we only included presymptomatic mutation carriers in this analysis who underwent Holter recording during follow-up. Holter recording was performed upon discretion of the treating physician, but is typically performed once every two years in these mutation carriers.

Statistical analysis

Data are expressed as mean ± standard deviation or median [interquartile range] as appropriate. Continuous variables were compared between two groups using an independent

samples t-test or Mann-Whitney U test as appropriate. Multiple groups were compared using one-way analysis of variance or Kruskal Wallis test as appropriate, with adjusted p-values by Bonferroni correction for multiple testing. Proportions were compared between groups using Chi-square test or Fisher's exact test as appropriate. Echocardiographic measurements that were significantly different between control subjects and presymptomatic mutation carriers were tested with univariable Cox regression analysis for prediction of VA among presymptomatic mutation carriers. The following pre-defined clinical variables were also tested with univariable Cox regression: age, sex, PVC count at baseline, presence of low QRS voltages at baseline and number of leads with T-wave inversions at baseline. The single strongest echocardiographic predictor (i.e. with lowest p-value in univariable analysis) was entered in multivariable analysis with other clinical variables that reached p<0.10 in univariable analysis. Hazards ratios (HR) are provided with 95% confidence intervals (CI). Two-tailed p-values of <0.05 were considered to indicate statistical significance. Statistical analyses were performed with IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, New York).

Results

Study population

Baseline characteristics are presented in **Table 1**. The final study population comprised 281 mutation carriers, of which 139 (50%) were classified into the presymptomatic stage, 91 (32%) into the arrhythmic stage and 51 (18%) into the structural stage. Control subjects had similar age and sex distribution as presymptomatic mutation carriers.

Clinical characteristics of presymptomatic mutation carriers

All presymptomatic mutation carriers were relatives of index patients who were screened as part of cascade family screening. The median age of presymptomatic mutation carriers was 33 [21-42] years and 59 (42%) were males. By design, none of them fulfilled current diagnostic criteria for ARVC or DCM at baseline and none of them had an ICD (11,23). All of them were classified as NYHA Class I. Nine presymptomatic mutation carriers (7%) had cardiac medication at baseline, which was eplerenone in five (trial medication, clinicaltrials.gov identifier: NCT01857856) an angiotensin-converting enzyme inhibitor in three and a beta-blocker in one.

Holter recordings were available in 128 presymptomatic mutation carriers (92%). The median number of PVCs in presymptomatic mutation carriers was 4 [1-55] per 24 hours.

ECGs were available in 133 presymptomatic mutation carriers (96%). T-wave inversions were not assessed in three because of an RBBB. Ten presymptomatic mutation carriers (8%) had T-wave inversions in more than one lead. Low QRS voltages were found in 16 presymptomatic mutation carriers (12%).

CMR studies were available in 78 presymptomatic mutation carriers (56%). LGE was present in 19 (24%) and was most often seen in the LV free wall (n=16, 21%), followed by hingepoint LGE (n=3, 4%) and apical LGE (n=1, 1%). RV LGE was seen in none of the presymptomatic mutation carriers.

Echocardiographic evaluation

Conventional measurements

Echocardiographic measurements are presented in **Table 2**. By conventional measurements, presymptomatic mutation carriers had similar LV and left atrial volumes and RV dimensions in comparison to control subjects. Conventional measurements for LV systolic and diastolic

function and RV systolic function in presymptomatic mutation carriers were also similar to control subjects.

Deformation imaging (global)

GLS was found to be lower in presymptomatic mutation carriers than in control subjects (19.9 ± 1.9% vs. 21.5 ± 1.8%, respectively, p<0.001). GLS was even lower in the arrhythmic stage (18.4 ± 2.1%, p<0.001) and in the structural stage (11.1 ± 4.0%, p<0.001). Accordingly, LVMD was higher in presymptomatic mutation carriers than in control subjects (31 [27-39] ms vs. 25 [20-30] ms, respectively, p<0.001) and even higher in the arrhythmic stage (47 [38-57] ms, p<0.001) and in the structural stage (62 [52-73] ms, p<0.001). RV deformation imaging was feasible in 67 presymptomatic mutation carriers (48%), but RVFWS was not significantly different in comparison to control subjects.

Deformation imaging (regional)

Rates of PSS in different LV segments are visualized in **Figure 2**. In presymptomatic mutation carriers, PSS was most pronounced in the apical segments, being present in 43 subjects (31%). Presence of apical PSS was even more frequent in the arrhythmic stage (n=59, 66%) and in the structural stage (n=50, 98%). In control subjects, PSS was never seen in the apical segments. Exact rates of PSS per segment are shown in **supplemental table 1**.

Characteristics of presymptomatic subjects with apical PSS compared to those without apical PSS are shown in **Table 3**. Age and sex were comparable between these groups, and also the conventional echocardiographic measurements were not different. Subjects with apical PSS had increased LVMD in comparison to subjects without apical PSS (40 [34-49] ms vs. 29 [25-34] ms, respectively, p<0.001). Subjects with apical PSS also tended to have low QRS voltages (p=0.064) and LGE (p=0.080) more frequently than

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subjects without apical PSS. However, it should be noted that LGE in the apical region was only seen in one subject with apical PSS (4%).

Follow-up of presymptomatic mutation carriers

Follow-up Holter recordings were available in 104 presymptomatic mutation carriers (75%). Presymptomatic subjects without follow-up Holter were typically subjects who had their baseline echocardiogram in the last three years of the inclusion period (30 out of 35). After a median follow-up duration of 3.2 [2.1-5.6] years, nonsustained VA occurred in 13 subjects (13%). Sustained VA was not observed in these subjects. The median nonsustained VA cycle length was 364 [274-430] ms, whereas the median VA duration was 4 [3-7] beats.

Results of Cox regression analysis are shown in **Table 4**. Of all echocardiographic variables that were tested in univariable analysis, presence of apical PSS was the strongest predictor of VA (p=0.018). Presence of apical PSS remained a significant predictor when it was entered in multivariable analysis together with male sex (multivariable HR: 5.11 [1.37 – 19.08], p=0.015).

Of the subjects with apical PSS at baseline with available follow-up (n=34), 10 developed VA (PPV=29% [95% CI: 21-40%]). Of the subjects without apical PSS at baseline with available follow-up (n=70), three developed VA (NPV=96% [95% CI: 89-98%]) (**Figure 3**). Follow-up duration was similar between presymptomatic subjects with and without apical PSS (p=0.986). The three subjects without apical PSS at baseline who developed VA during follow-up were typically subjects with a long follow-up duration (characteristics shown in **supplemental table 2**).

Discussion

The key findings of this study are that (i) echocardiographic deformation imaging reveals global and in particular regional apical mechanical alterations in presymptomatic PLN p.Arg14del mutation carriers and that (ii) presymptomatic mutation carriers with abnormal apical deformation patterns appear to be at higher risk for VA. These findings imply that echocardiographic deformation characteristics may be used for early disease stage classification (**Central Illustration**), and have potential to improve clinical management of presymptomatic mutation carriers.

Early detection

During the systolic phase of the cardiac cycle, calcium is released from the sarcoplasmic reticulum into the cytosol which triggers contraction of the cardiomyocyte. During diastole, calcium should be transported back into the sarcoplasmic reticulum, which is essential for rapid cardiomyocyte relaxation. In patients with the PLN p.Arg14del mutation, calcium handling is disturbed (30). It is assumed that the mutated PLN protein inhibits calcium uptake in the sarcoplasmic reticulum, which primarily leads to high concentrations of calcium in the cytosol during diastole (5).

In our study we observed mechanical alterations in PLN p.Arg14del mutation carriers who are in very early stages of disease, in particular regional alterations. PSS in the LV apex was observed in approximately one third of presymptomatic mutation carriers in our cohort and in none of the control subjects. We speculate that this early diastolic phenomenon (occurring predominantly during the isovolumetric relaxation phase) is a sign of impaired relaxation due to diastolic calcium overload in cardiomyocytes. Importantly, we found that presence of apical PSS in presymptomatic mutation carriers was not associated with presence of LGE in the apical region, which supports the concept that apical PSS represents an (electro-)mechanical substrate that differs from early myocardial fibrosis (24). This is also

supported by prior studies that showed PSS in patients with long QT syndrome, considering that long QT syndrome is characterized by prolonged action potential duration without presence of any structural heart disease (31,32).

It remains unknown why PSS is confined to the LV apex during early stages of disease in PLN p.Arg14del mutation carriers. A previous study by Sengupta *et al.* using invasive measurements in healthy pigs suggested that the apical region is the last region to be repolarized in the LV and also the last one to start with diastolic lengthening (33). This might explain why diastolic calcium imbalance becomes first apparent in the LV apex in PLN p.Arg14del carriers. However, the normal sequence of local ventricular repolarization is still largely unknown and therefore this hypothesis remains to be further investigated.

Disease stage classification

In this study we could subdivide presymptomatic mutation carriers into two distinct disease stages (**Central Illustration**). Mutation carriers with normal deformation patterns in the LV apex were found to be at very low risk of developing any VA within three years and were therefore deemed to be in a concealed stage of disease. In contrast, mutation carriers with PSS in the apex appeared to be at higher risk of developing nonsustained VA during follow-up. Since nonsustained VA was previously found to precede occurrence of sustained VA, we consider apical PSS to be an important subclinical sign of disease (13). In contrast to regional deformation, GLS was not found to be associated with occurrence of VA in this study.

It is currently recommended to perform repeated cardiac screening in mutation carriers every 2-3 years in case of normal cardiovascular tests (11,12). Follow-up protocols are usually intensified from late adolescence onwards because of the age-related penetrance of disease (3,13,14). The results of our study suggest that deformation imaging can be used to further tailor follow-up protocols for presymptomatic PLN p.Arg14del mutation carriers.

More specifically, since mutation carriers with apical PSS appear to be at higher risk of developing VA, follow-up frequency should be higher in these individuals compared to mutation carriers with normal deformation patterns in the LV apex. Future longitudinal studies should be conducted to determine optimal follow-up intervals in these mutation carriers. Moreover, if medical treatment becomes available for presymptomatic mutation carriers, interventional studies investigating deformation imaging as an early tool for treatment selection would also be of interest.

Limitations

For this explorative study, we considered PSS to be present when the post-systolic index was ≥10% in a segment. However, prolonged contraction duration with less pronounced PSS may also be of clinical relevance in PLN p.Arg14del mutation carriers. Future studies should therefore aim to optimize classification of deformation patterns, for example with machine learning algorithms.

Due to the relatively short follow-up duration, only nonsustained VA was observed in presymptomatic mutation carriers. The predictive value of apical PSS for life-threatening VA remains to be investigated in studies with longer follow-up. Furthermore, the effect of other clinical variables may be underestimated due to relatively small number of events. These variables should therefore not be neglected on the basis of our results.

Apical LGE was not observed in subjects who had apical PSS. However, absence of LGE does not exclude presence of subtle structural myocardial changes. More comprehensive CMR techniques, such as T1 mapping, would shed more light on the possible underlying substrate for mechanical dysfunction.

Conclusion

Echocardiographic deformation imaging reveals global and regional mechanical alterations in PLN p.Arg14del mutation carriers before arrhythmic symptoms and overt structural disease. Presymptomatic mutation carriers with normal deformation patterns in the LV apex are at low risk of developing VA within three years, whereas mutation carriers with apical PSS appear to be at higher risk. Future studies with longer follow-up should be conducted to determine the added value of deformation imaging with regard to clinical management of presymptomatic mutation carriers.

PERSPECTIVES

<u>Competency in medical knowledge</u>: The present study demonstrates that echocardiographic deformation imaging reveals mechanical alterations in presymptomatic phospholamban p.Arg14del mutation carriers. By assessing deformation patterns in the left ventricular apex, presymptomatic mutation carriers can be reclassified into two distinct disease stages: the concealed stage and the subclinical stage. The proposed disease stage classification provides opportunities to improve early management in this challenging group of at-risk individuals.

<u>Translational outlook</u>: To elucidate whether apical post-systolic shortening represents an early electro-mechanical substrate in presymptomatic mutation carriers, future studies should correlate deformation characteristics with (non-)invasive electrophysiological investigations. It would be of particular interest to study whether mutation carriers with subclinical apical post-systolic shortening develop ventricular arrhythmias originating from the apex. Moreover, more comprehensive characterization of regions with post-systolic shortening with cardiovascular magnetic resonance imaging (in particular T1 mapping) would also be of interest.

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Figure legends

Figure 1: Inclusion flowchart
PLN p.Arg14del mutation carriers were derived from a national database. Mutation carriers were eligible for this study if they had an available echocardiogram of sufficient quality. The final study population comprised 281 mutation carriers, who were classified to the presymptomatic stage (n=139), the arrhythmic stage (n=91) or the structural stage (n=51). LBBB = left bundle branch block, LVEF = left ventricular ejection fraction, PLN = phospholamban, PVC = premature ventricular complex, VA = ventricular arrhythmia.

Figure 2: Predilection sites of post-systolic shortening in different subgroups
LV models are presented for different subgroups. The colors depict the amount of subjects with PSS in a particular segment. PSS was already found in presymptomatic mutation carriers and was most pronounced in the apical segments. A = anterior, AS = anteroseptal, I = inferior, L = lateral, S = septal, P = posterior, PSS = post-systolic shortening.

Figure 3: Prediction of ventricular arrhythmia in presymptomatic mutation carriers
On the basis of presence of PSS in the LV apex, presymptomatic mutation carriers could be subdivided in two groups. Subjects without PSS (i.e. concealed stage) were at low risk of developing VA, whereas subjects with PSS (i.e. subclinical stage) were at higher risk of developing nonsustained VA. NPV = negative predictive value, PLN = phospholamban, PPV = positive predictive value, VA = ventricular arrhythmia.

Central Illustration: Disease classification on the basis of myocardial deformation characteristics
On the basis of presence of PSS in the LV apex, presymptomatic mutation carriers could be reclassified into the concealed stage (no apical PSS) or into the subclinical stage (presence of apical PSS). Subjects in the concealed stage had normal values of LVMD and GLS, whereas subjects in the subclinical stage had increased LVMD and occasionally impaired GLS. In symptomatic subjects (arrhythmic or structural stage), myocardial deformation characteristics were even more impaired. GLS = global longitudinal strain, LVEF = left ventricular ejection fraction, LVMD = left ventricular mechanical dispersion, PSS = post-systolic shortening, RVEF = right ventricular ejection fraction.

Tables

Table 1: Baseline characteristics

	Controls (n=70)	Presymptomatic (n=139)	Arrhythmic (n=91)	Structural (n=51)
Age (years)	33 [25-44]	33 [21-42]	49 [40-57]*	51 [41-64]*
Male sex	26 (37)	59 (42)	43 (47)	22 (43)
BSA (m2)	1.8 [1.7-1.9]	1.9 [1.8-2.0]	2.0 [1.8-2.1]*	1.9 [1.8-2.1]
Proband	-	0 (0)	25 (27)	33 (65)
ARVC Dx	-	0 (0)	29 (32)	21 (41)
DCM Dx	-	0 (0)	0 (0)	43 (84)
VA history	-	0 (0)	57 (63)	40 (78)
ICD	-	0 (0)	26 (29)	34 (67)
Cardiac medication	-	9 (7)	53 (58)	50 (98)
ACE-i/ARB	-	3 (2)	24 (17)	38 (27)
Anti-arrhythmic	-	0 (0)	11 (8)	32 (23)
Beta-blocker	-	1 (1)	29 (21)	61 (44)
Diuretic	-	5 (4)	11 (8)	42 (30)
LVEF (%)	60 ± 4	58 ± 4	55 ± 5*	35 ± 10*
NYHA Class ≥II	-	0 (0)	6 (7)	26 (51)
PVC count (n/24h)	-	4 [1-55]	1400 [814-3849]	2652 [1111-5135]
TWI in >1 lead	-	10/130 (8)	41/86 (48)	20/47 (43)
Low QRS voltages	-	16/133 (12)	36/89 (41)	37/50 (74)
LGE	-	19/78 (24)	23/42 (55)	6/7 (86)

Values are presented as mean ± SD, median [IQR] or n (%) as appropriate. An asterisk (*) indicates a statistical significant difference in comparison to control subjects (i.e. Bonferroni adjusted $p < 0.05$). ACE-i = angiotensin-converting enzyme inhibitor, ARB = angiotensin II

receptor blocker, ARVC = arrhythmogenic right ventricular cardiomyopathy, BSA = body surface area, DCM = dilated cardiomyopathy, Dx = diagnosis, ICD = implantable cardioverter defibrillator, LGE = late gadolinium enhancement, LVEF = left ventricular ejection fraction, NYHA = New York Heart Association, PVC = premature ventricular complex, TWI = T-wave inversion, VA = ventricular arrhythmia.

Table 2: Echocardiographic measurements

	Controls (n=70)	Presymptomatic (n=139)	Arrhythmic (n=91)	Structural (n=51)
LVEDV (ml/m2)	54.2 [46.7-61.6]	53.8 [46.7-60.3]	55.6 [48.5-63.0]	76.2 [62.4-102.3]*
LVESV (ml/m2)	21.4 [18.4-25.2]	21.3 [17.6-25.6]	25.5 [20.4-29.9]*	47.7 [36.5-72.2]*
LVEF (%)	59.8 ± 4.4	58.3 ± 4.2	54.5 ± 5.2*	35.0 ± 9.6*
RVOT-PLAX (mm/m2)	15.4 [14.2-16.7]	14.9 [13.8-16.4]	16.9 [15.4-17.9]*	19.4 [16.6-23.1]*
RVOT-PSAX (mm/m2)	16.0 [14.8-17.6]	15.8 [14.1-17.5]	17.3 [15.8-18.6]	20.5 [17.0-22.8]*
RVEDA (cm2/m2)	9.4 [8.4-10.5]	9.4 [8.3-10.4]	10.1 [8.5-12.0]	13.4 [11.5-15.8]*
RVESA (cm2/m2)	4.8 [4.4-5.9]	5.0 [4.3-5.8]	6.1 [4.8-7.3]*	10.1 [7.6-11.7]*
RV FAC (%)	46.8 ± 4.8	46.1 ± 5.5	41.3 ± 7.9*	28.8 ± 7.6*
LAVI (ml/m2)	28.7 ± 6.8	27.7 ± 6.2	30.4 ± 7.7	41.3 ± 13.3*
E-wave velocity (cm/s)	81.0 [70.6-90.3]	78.7 [66.0-89.0]	64.5 [51.3-80.0]*	61.2 [50.1-74.3]*
A-wave velocity (cm/s)	44.8 [39.4-56.8]	51.0 [42.0-59.0]	53.3 [39.0-65.0]	49.9 [35.5-62.3]*
E-wave DT (ms)	175 [161-199]	181 [155-203]	203 [176-232]*	157 [141-202]*
Avg e' velocity (cm/s)	14.5 [11.5-15.8]	13.5 [10.5-16.0]	9.5 [8.0-12.0]*	6.0 [4.5-8.0]*
E/e' ratio	5.9 [4.9-6.4]	5.9 [5.1-6.9]	6.3 [5.3-7.8]	9.9 [6.9-11.8]*
GLS (%)	21.5 ± 1.8	19.9 ± 1.9*	18.4 ± 2.1*	11.1 ± 4.0*
LVMD (ms)	25 [20-30]	31 [27-39]*	47 [38-57]*	62 [52-73]*
RVFWS (%)	26.6 ± 3.7	25.3 ± 3.6	21.8 ± 4.2*	14.1 ± 5.5*

Values are presented as mean ± SD, median [IQR] or n (%) as appropriate. An asterisk (*) indicates a statistical significant difference in comparison to control subjects (i.e. Bonferroni adjusted $p < 0.05$). Avg = average, DT = deceleration time, FAC = fractional area change, GLS = global longitudinal strain, LAVI = left atrial volume index, LVEDV/LVESV = left ventricular end-diastolic/end-systolic volume, LVEF =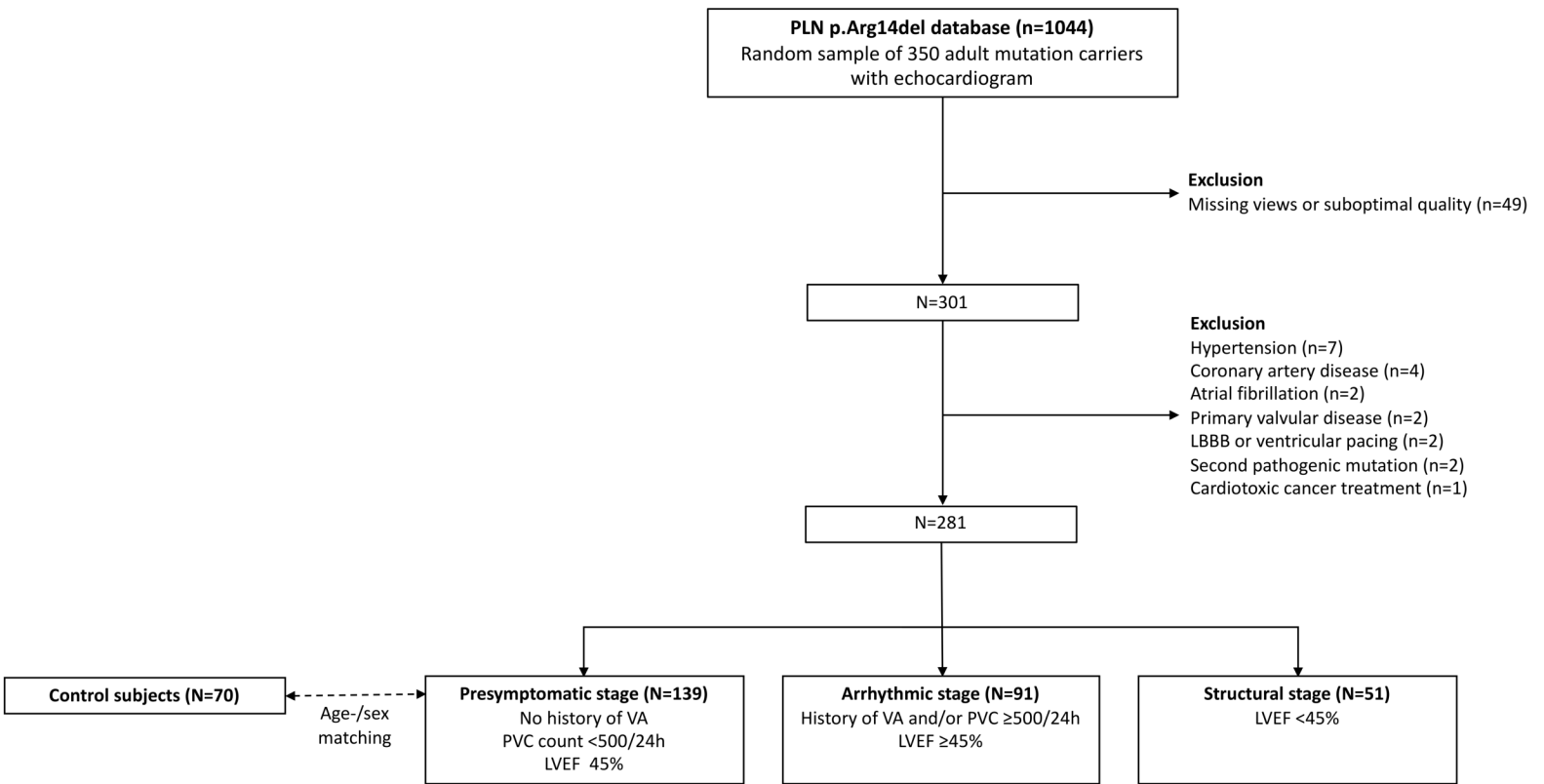 left ventricular ejection fraction, LVMD = left ventricular mechanical dispersion, RV = right ventricle/ventricular, RVEDA/RVESA = right ventricular end-diastolic/end-systolic area, RVFWS = right ventricular free-wall strain, RVOT-PLAX/PSAX = right ventricular outflow tract in parasternal long axis/short axis view.

Table 3: Characteristics of presymptomatic subjects with and without apical PSS

	Apical PSS + (n=43)	Apical PSS – (n=96)	p-value
Age (years)	33 [23-41]	33 [20-43]	0.607
Male sex	22 (51)	37 (39)	0.164
LVEDV (ml/m2)	54.0 [49.4-61.4]	53.3 [45.9-59.9]	0.353
LVESV (ml/m2)	21.4 [19.4-26.9]	20.4 [17.4-24.7]	0.284
LVEF (%)	57.5 ± 4.7	58.8 ± 4.0	0.103
RVEDA (cm2/m2)	9.4 [8.3-10.4]	9.5 [8.7-10.4]	0.785
RVESA (cm2/m2)	5.1 [4.1-5.8]	5.0 [4.4-5.8]	0.709
RV FAC (%)	46.1 ± 5.3	46.1 ± 5.7	0.951
GLS (%)	19.5 ± 2.0	20.1 ± 1.8	0.089
LVMD (ms)	40 [34-49]	29 [25-34]	<0.001*
RVFWS (%)	24.4 ± 3.9	25.8 ± 3.3	0.119
PVC count (n/24h)	5 [2-155]	4 [1-37]	0.172
Low QRS voltages	8/40 (20)	8/93 (9)	0.064
TWI in >1 lead	5/39 (13)	5/91 (6)	0.165
Apical LGE	1/23 (4)	0/55 (0)	0.295
Any LGE	9/23 (39)	1/55 (18)	0.080

Values are presented as mean ± SD, median [IQR], or n (%) as appropriate. An asterisk (*) indicates a statistical significant difference between the two groups (p<0.05). FAC = fractional area change, GLS = global longitudinal strain, LGE = late gadolinium enhancement, LVEDV/LVESV = left ventricular end-diastolic/end-systolic volume, LVEF = left ventricular ejection fraction, LVMD = left ventricular mechanical dispersion, PSS = post-

systolic shortening, PVC = premature ventricular complex, RV = right ventricle/ventricular, RVEDA/RVESA = right ventricular end-diastolic/end-systolic area, RVFWS = right ventricular free-wall strain, TWI = T-wave inversion.

Table 4: Prediction of ventricular arrhythmia in presymptomatic mutation carriers

Variable	VA+ (n=13)	VA- (n=91)	Univariable HR [95% CI]	p-value	Multivariable HR [95% CI]	p-value
Echocardiographic variables						
GLS (%)	19.9 ± 1.6	20.0 ± 1.9	0.98 [0.67 – 1.47]	0.953		
LVMD (ms)	39 [30-48]	31 [26-37]	1.06 [1.00 – 1.11]	0.036**		
Apical PSS	10 (77)	24 (26)	4.97 [1.32 – 18.72]	0.018**	5.11 [1.37 – 19.08]	0.015**
Other clinical variables						
Age (years)	33 [21-39]	33 [22-42]	1.02 [0.98 – 1.06]	0.401		
Male sex	9 (69)	34 (37)	2.95 [0.91 – 9.65]	0.073*	3.17 [0.96 – 10.47]	0.059*
PVC count (n/24h)	5 [2-105]	4 [1-39]	1.00 [0.99 – 1.01]	0.972		
Low QRS voltages	4/13 (31)	9/88 (10)	1.99 [0.57 – 7.01]	0.282		
Leads with TWI (n)	0 [0-0]	0 [0-1]	0.78 [0.28 – 2.21]	0.643		

Values are presented as mean ± SD, median [IQR], or n (%) as appropriate. A single asterisk (*) indicates a p-value <0.10, whereas a double asterisk (**) indicates a p-value <0.05. The single strongest echocardiographic variable (apical PSS) was tested in multivariable Cox regression

analysis with other clinical variables that reached p<0.10 (*) in univariable analysis (only male sex). CI = confidence interval, GLS = global longitudinal strain, HR = hazard ratio, LVMD = left ventricular mechanical dispersion, PSS = post-systolic shortening, PVC = premature ventricular complex, TWI = T-wave inversion, VA = ventricular arrhythmia.

PLN p.Arg14del database (n=1044)
Random sample of 350 adult mutation carriers
with echocardiogram

Exclusion
Missing views or suboptimal quality (n=49)

N=301

Exclusion
Hypertension (n=7)
Coronary artery disease (n=4)
Atrial fibrillation (n=2)
Primary valvular disease (n=2)
LBBB or ventricular pacing (n=2)
Second pathogenic mutation (n=2)
Cardiotoxic cancer treatment (n=1)

N=281

Control subjects (N=70)

Age-/sex
matching

Presymptomatic stage (N=139)
No history of VA
PVC count <500/24h
LVEF 45%

Arrhythmic stage (N=91)
History of VA and/or PVC ≥500/24h
LVEF ≥45%

Structural stage (N=51)
LVEF <45%

Subjects with PSS
- <10%
- ≥10%, <20%
- ≥20%, <30%
- ≥30%, <40%
- ≥40%

Control subjects

Presymptomatic stage
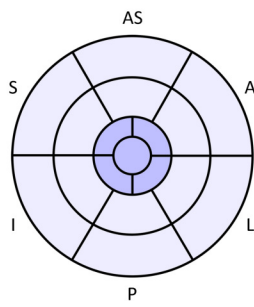
Arrhythmic stage
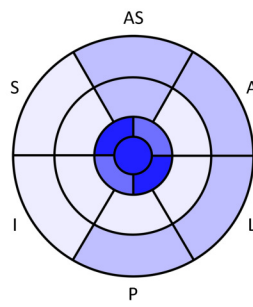
Structural stage
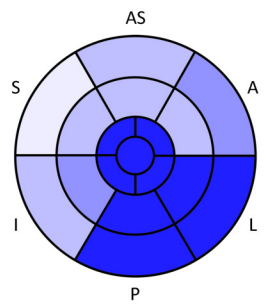
Presymptomatic PLN p.Arg14del mutation carriers

No apical PSS
(n=96)
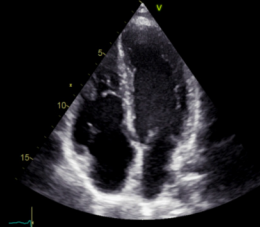
Apical PSS
(n=43)
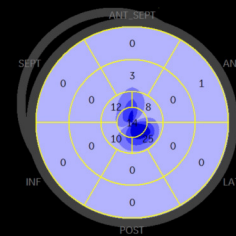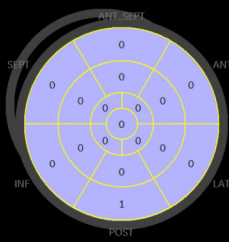
Concealed stage

Subclinical stage

Follow-up Holter (N=70)

Follow-up Holter (N=34)
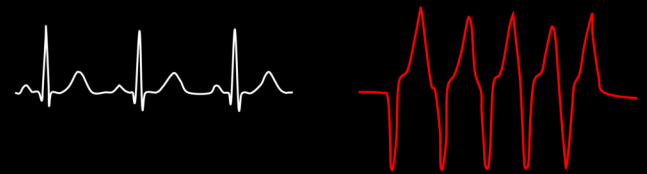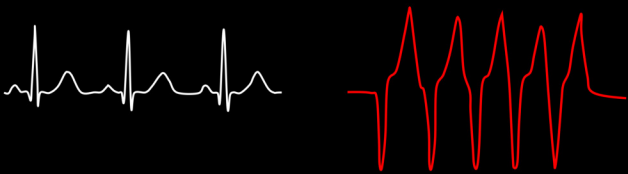
no VA (N=67)

VA (N=3)

no VA (N=24)

VA (N=10)

NPV = 96%

PPV = 29%

PLN p.Arg14del mutation carriers

Presymptomatic

Symptomatic

Concealed stage

Subclinical stage

Arrhythmic stage
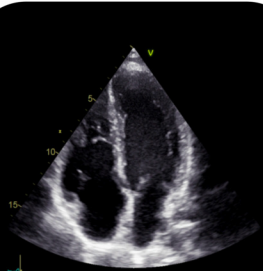
Structural stage

LVEF normal
RVEF normal
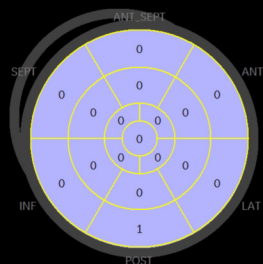
No PSS
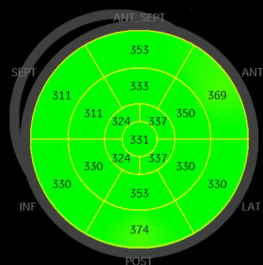
LVMD normal

GLS normal
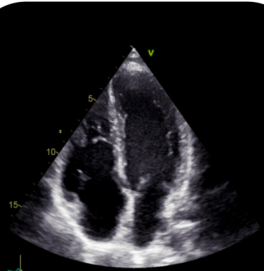
LVEF normal
RVEF normal
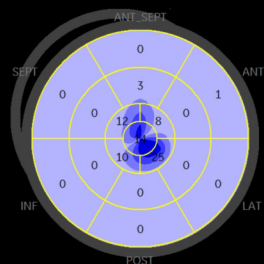
Apical PSS
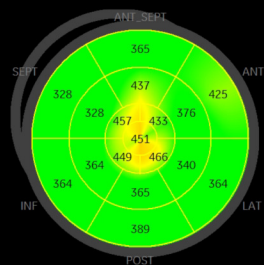
LVMD ↑
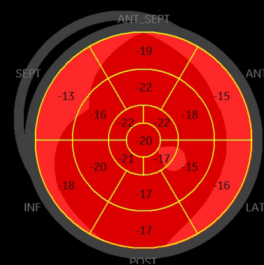
GLS normal or ↓
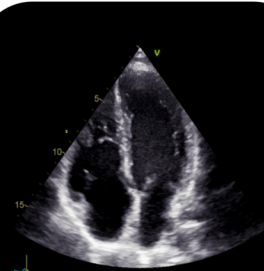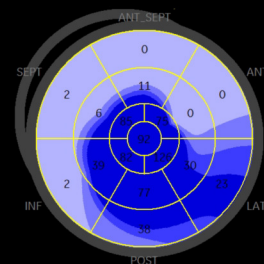
LVEF normal
RVEF normal or ↓

PSS ↑↑

LVMD ↑↑

GLS normal or ↓(↓)

LVEF ↓
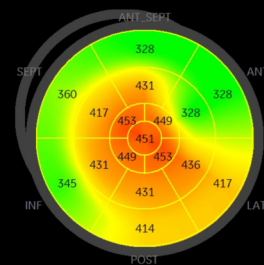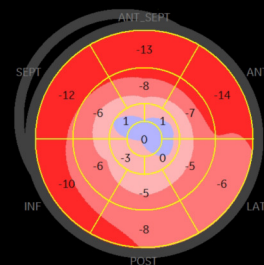RVEF ↓

PSS ↑↑↑

LVMD ↑↑↑

GLS ↓↓↓